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Plant diversity changes in a nature reserve: a probabilistic sampling method for quantitative assessments

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Abstract

Species pool conservation is critical for the stability of ecosystem processes. However, climate and land use changes will likely affect biodiversity, and managers of protected areas are under increasing pressure to monitor native species diversity changes by approaches that are scientifically sound and comparable over time. Here we describe a plant diversity monitoring system in use since 2002 in the "Montagna di Torricchio" Nature Reserve (LTER_EU_IT_033), a Central Apennines representative area of 317 ha, most of which is under strict protection. The aim of this paper was to assess changes in plant species richness over time and to deduce the patterns of species assemblage. The monitoring system was based on a probabilistic sampling design representative of the different physiognomic vegetation types occurring in the Reserve. A total of 34 plots (10×10m) were sampled in 2002, 2003 and 2015, and their species presence/absence and relative coverage were estimated. Repeated measure ANOVA was used to test for plot-level and ecosystem-based changes in species richness along the study period. Temporal nestedness and temporal turnover metrics were used to assess patterns of species' compositional changes. The results

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showed significantly different levels of species richness depending on the year, with the lowest value in 2003, probably linked to extreme drought events. Forest systems were comparatively stable, demonstrating the capacity to buffer interannual climate variability. Regarding compositional changes along the entire period (2002–2015), we found random patterns of both temporal nestedness and turnover, indicating stability in species composition. However, we also showed the contemporary occurrence of species loss and species replacement processes, considering the dry year 2003, a finding which should be further explored through fine-scale studies to unravel mechanisms of community assembly under drought. The use of a probabilistic sampling design representative of the different physiognomic vegetation types proved to be advantageous in monitoring the Nature Reserve vegetation and collecting reliable quantitative information. This data, in turn, provides the basis for improvements in management practices and proposed adaptation measures.

Keywords
compositional changes, monitoring, LTER, species richness, temporal nestedness, temporal turnover

Introduction

Species pool conservation is critical to the stability of ecosystem processes (Loreau et al. 2001). However, climate and land use changes will likely affect biodiversity at local to landscape scale (Sala et al. 2000; Thomas et al. 2004). Despite commitments by the international community to halt biodiversity decline, conservation efforts targeting plant diversity are often hampered by the lack of suitable data for prioritising conservation action (Darbyshire et al. 2017). In particular, one of the main debates in the field of conservation biology is the assessment of results obtained in efforts to meet conservation goals fixed by international treaties, evaluating the efficiency of existing protected areas (Vellak et al. 2009; Capotorti et al. 2012; Pringle 2017). In Europe, the implementation of the EU Directives 09/147/CE (Birds Directive) and 92/43/CE (Habitats Directive) in the Natura 2000 network has been the main pillar for biodiversity conservation. Here, biodiversity monitoring is legally binding (i.e. articles 11 and 17 of the Habitats Directive, Bock et al. 2005). In this context, the assessment of species diversity is crucial, since this fundamental property of ecological communities can serve as a parameter for comparing assemblages in time and space, independently from species identities (Chiarucci et al. 2008 and references therein). Managers of protected areas are thus under increasing pressure to monitor species diversity changes by approaches that are scientifically sound and comparable over time. Many monitoring approaches are highly subjective in the choice of where to position observational units, thus violating the assumptions of randomness, independence and known probability (Chiarucci 2007). On the contrary, the selection of observational units driven by a probabilistic approach permits sound generalisation (Lájer 2007).

Global change trends are undoubtedly producing effects on species diversity in different ecosystems (e.g. van Vuuren et al. 2006; Chelli et al. 2017; Rogora et al. 2018). In addition, there is growing consensus on the major impact of interannual climate variability (Grime et al. 2008; Cleland et al. 2013) and extreme weather events on plant communities (Jentsch et al. 2007), including seasonal effects (Chelli et al. 2016) e.g., extreme drought (Wellstein et al. 2017). In order to assess these changes in plant

communities, species inventories and long-term monitoring of permanent plots are essential. Among different climatic parameters, interannual rainfall variation seems to have the most significant effect on plant species richness, composition and functioning (Adler and Levine 2007), especially in ecosystems where water is a primary limiting resource (Chelli et al. 2017). Regarding the direction of changes, resource supply influences species richness via its effects on the competitive interactions and growth rates of species already present in the local community (Adler and Levine 2007). Traditional explanations foresee that decreasing resources may increase species richness due to the reduced competitive strength of the dominant species (Grime 1979), or instead may reduce species richness by allowing a lower number of species to tolerate the environment (Preston 1962, Wright 1983). In Mediterranean-type ecosystems Chiarucci et al. (2003) found a negative effect of drought on species richness. However, it has been demonstrated that at relatively small geographic or local scales, different factors provide a spatial and functional buffer against the uncertainty of interannual climate variability and short-term extremes, as well as soil properties and heterogeneity (Jentsch and Beierkuhnlein 2008; Fridley et al. 2011), complex topography (Loarie et al. 2009), ecosystem structure (e.g. canopy cover in forests, Chen et al. 1999) and plant phenotypic plasticity (Wellstein et al. 2013).

In addition to climate, dynamic processes deriving from land use changes can produce significant changes in species richness and composition both in grasslands (Catorci et al. 2013; Giarrizzo et al. 2017) and forests (Bartha et al. 2008), due to competitive exclusion of subordinate species (Wellstein et al. 2014; Tardella et al. 2017) or to shifts in abiotic conditions (Campetella et al. 2011).

In addition to species richness, patterns of species assemblages over time are also particularly significant for understanding ecological processes (Kopecký et al. 2013). In this context, analysis of temporal beta diversity (variation of the species composition of plant assemblages over time) may reflect two different phenomena: species turnover and nestedness of assemblages. They result from two antithetic processes (Gaston and Blackburn 2000), namely, species replacement resulting in species turnover and species loss resulting in nestedness (Baselga 2010). Nestedness occurs when species-poor assemblages represent a subset of more species-rich communities (Patterson and Atmar 1986), and thus difference in species richness is a necessary condition for nestedness (Ulrich and Almeida-Neto 2012). According to Elmendorf and Harrison (2009), nestedness can occur when most species respond similarly to interannual variation in conditions. On the contrary, turnover can occur when different sets of species are present in different years, either because of different resource requirements (due to different environmental conditions) or as a result of competitive exclusion.

Despite their potential, such metrics have been rarely used in diachronic datasets (but see Elmendorf and Harrison 2009 in grasslands; Kopecký et al. 2013; Canullo et al. 2017, in forest ecosystems) and never used in monitoring programmes encompassing different ecosystems.

Temporal analyses of this kind could be affected by imperfect detection of species, such as when species are overlooked, misidentified, or when assessments contain errors, and this can lead to 'pseudoturnover' (Morrison 2016). Therefore, a stand-

ard, replicable and statistically representative method is suggested to avoid one of the most common causes of unreliability in the comparison of diversity assessments. To assess and compare biodiversity over time, a random sampling design is recommended (Magurran 2004).

The aim of this paper was to assess changes in vascular plant species richness over time and to deduce the patterns of species assemblage through a monitoring system based on a standard probabilistic sampling design. The "Montagna di Torricchio" LTER site (LTER_EU_IT_033), which includes typical Mediterranean-montane systems, was used as the study area. The study site is part of the Italian "Important Plant Areas" identified by Blasi et al. (2011) and is included in the Natura 2000 network (IT5330022 - Montagna di Torricchio).

In particular, we hypothesised that:

(a) species richness at plot level significantly changes over time according to inter-annual climate variability (including extreme climatic events, e.g. drought) and/or dynamic processes deriving from land-use change;
(b) forest ecosystems buffer climate variability and show a certain stability in terms of species richness over time, compared to grasslands. Finally, we explored the patterns of species compositional changes over time (in terms of both temporal nestedness and temporal turnover) along the entire monitoring period and for each couple of years of relevés. Given the scarcity of approaches exploring patterns of compositional changes in different ecosystems, our findings can be useful to support future hypotheses for monitoring programmes in complex landscapes.

Methods

Study area

The "Montagna di Torricchio" Nature Reserve is located in the Central Apennines, Italy (+130050E, +425740N, WGS84; Fig. 1a). It covers an area of 317 ha along two north- and south-facing slopes of a SW-NE oriented valley, spanning an elevation from 820 to 1490 m a.s.l. Mean annual precipitation reaches 1250 mm and mean annual temperature is around 11 °C. Jurassic-Cretaceous limestone prevails in the area (Kwiatkowski and Venanzoni 1994). The Reserve hosts different ecosystems, including xeric and mesic grasslands, grasslands with scattered shrubs, sub-Mediterranean hop-hornbeam (*Ostrya carpinifolia*) and ash (*Fraxinus ornus*) open woods, and beech (*Fagus sylvatica*) forests. The area has been under strict protection since 1970 with legacies for nature conservation, research and education. The management plan is devoted to protecting the natural processes that have been underway since the 1960s, when traditional management began to be abandoned, leading to natural succession. Previously, the grasslands had been managed as sheep-pastures, and forests had been coppiced with a rotation cycle of about 25 years (Campetella et al. 2016).

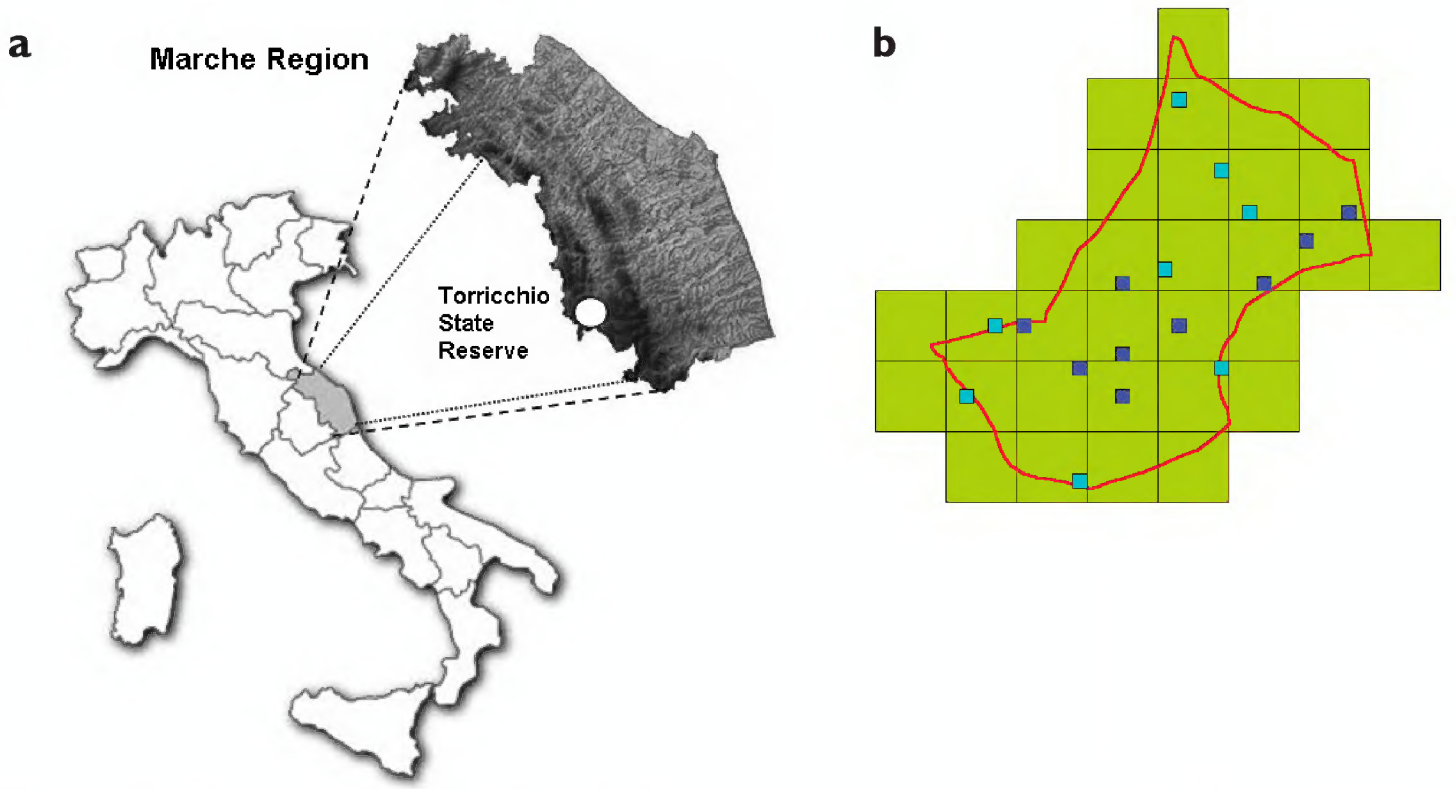

Figure 1. a Location of the "Montagna di Torricchio" Nature Reserve (LTER_EU_IT_033) in the Central Apennines, Marche Region, Italy (From: Wellstein et al. 2014) **b** Representation of the sampling system: the 25 ha grid was superimposed on the reference area, with the 17 macroplots randomly selected (each containing a cluster of four 10×10 m plots); the dark blue plots contain the clusters lying within the effective area of the Reserve, which is the subject of the present paper.

Sampling design

Based on a multi-scalar sampling protocol adopted to assess plant biodiversity (originally including three grain-levels: 1 m^2, 100 m^2 and 10,000 m^2, see Baffetta et al. 2007, Santi et al. 2010), the information on vascular plant diversity was collected at the 100 m^2 scale, 10×10 m (hereafter referred to as plot). The probabilistic sampling design was constructed on the Regional Technical Maps 325010 (1:10,000) georeferenced through the UTM (ED50) coordinates. The map was divided into a 500×500 m grid covering a reference surface for the Nature Reserve, resulting in 17 cells, and one macroplot of 100×100 m was randomly selected on each cell. A cluster of four random points, identified as the SW vertex of the corresponding 10×10 m plots, was generated within each single macroplot. Only the nine clusters fully included in the Nature Reserve were considered (Fig. 1b), located between 1,000 and 1480 m a.s.l. at both S- and N-facing slopes, encompassing xeric grasslands with *Bromus erectus*, *Sesleria nitida*, *Asperula purpurea*, *Brachypodium rupestre*, different successional stages (with shrubs as *Cytisus sessilifolius*, *Juniperus communis*, *Rosa* sp.), sparse thickets and forest communities dominated by *Fagus sylvatica*. Each plot was identified in the field, georeferenced (error ± 10m) and materialised with poles at the corners. Surveys were performed in the summer of 2002, with resampling in 2003 and 2015. Overall, 36 plots occurred in the Reserve; however, two of them were not considered because of problems with plot identification in the field, resulting in a total of 34 plots sampled. Field relevés

were performed to record the species composition and the relative structure of the vascular plant communities, by visual assessment of the abundance (Braun-Blanquet 1964). Further factors that helped us avoid overlooking or misidentifying species were our reference to a standard protocol and a common taxonomic source (Pignatti 1982), continuously updated knowledge of the species pool of the "Montagna di Torricchio" Nature Reserve, the availability of a dedicated section of the Herbarium CAME at the University of Camerino, and the assistance of specialists (as recommended by common international references; cf. Canullo et al 2013). Sintetic climatic data (yearly and seasonally aggregated) were obtained by yearly reports from the Agency for Agro-food Sector Services of the Marche Region (ASSAM).

Data analysis

Changes in species richness over time

The changes in the mean number of species in the study period, considering the entire dataset and the two main vegetation types separately (grasslands, including grasslands with shrubs, n = 22; forest habitats, n = 12), were tested using repeated-measures ANOVA. The Bonferroni post-hoc test was used to assess differences in species richness among the three study years.

Temporal nestedness analysis

In order to assess the species nestedness that occurred among the three survey periods (2002, 2003, 2015), we used temporal nestedness analysis (TNA). We compared the observed temporal nestedness (TN) between different survey periods (2002 vs 2003, 2003 vs 2015 and 2002 vs 2015) with the distribution of 999 TN values generated by random reshuffling of the survey period between temporally paired samples. More specifically, TNA is based on a comparison of the observed temporal nestedness between subsequent surveys from the same sample and the nestedness of the same subsequent surveys whose species configuration was randomly reshuffled according to species presence/absence collected in the field. In detail, (a) we calculated the Nestedness measure based on Overlap and Decreasing Fill for sites (NODFsites; Almeida-Neto et al. 2008), between each pair of the temporally sorted old and recent samples, then (b) we averaged these pairwise NODFsites values into a TN value of the whole data set and, finally (c) we compared the observed TN between the two selected survey periods to the distribution of 999 TN values generated by the randomisation procedure. For further details related to this methodology, please refer to Kopecký et al. (2013).

Temporal turnover analysis

To quantify the species temporal turnover (TT) that occurred among the three survey periods (2002, 2003, 2015), we used the Simpson dissimilarity index (βsim) based on

pairwise comparison (Baselga 2010; Canullo et al. 2017). Baselga (2010) described βsim as one of the components of the total beta diversity. Therefore the turnover analysis was calculated as follows:

$$\beta sim = \min(b,c)/[a+\min(b,c)]$$

where *a* is the number of species common to both plots, *b* is the number of species that occur in the first plot but not in the second and *c* is the number of species that occur in the second plot but not in the first (Baselga 2010). We used the same randomisation method as adopted for the TNA.

All statistical analyses were performed with R, version 2.14.1. In particular, the following R packages were used: the *stats* package for repeated measures ANOVA; the *betapart* package (function beta.pair) for the Simpson dissimilarity index calculation; the *vegan* package (function nestednodf) for temporal nestedness analysis.

Results

Species richness

Overall, 345 species were sampled during the monitoring period. The lowest number of species was recorded during 2003 (210 species), while 2015 showed the highest number of species (271 species). At plot level, species richness significantly differed among years (Sum of squares = 2811.78, df = 2, F = 24.69, $p < 0.001$, Fig. 2a). Considering the two vegetation macro-types separately, grasslands showed significantly different levels of species richness among the three years (Sum of squares = 2366.20, df = 2, F = 28.43, $p = 0.001$, Fig. 2b), while in forests we did not note significant changes in species richness at the plot level (Sum of squares = 555.38, df = 2, F = 3.21, $p = 0.060$, Fig. 2b).

Species compositional changes

The observed TN value along the entire period (2002–2015) did not differ significantly from the randomly generated assemblages (TN = 11.07, SES = -0.95, $p > 0.05$, Fig. 3a). On the contrary, the species assemblages in the year 2003 were temporally nested subsets of those from the year 2002 (TN = 31.28, SES = 4.36, $p = 0.001$, Fig. 3b), while in the comparison 2003–2015 the observed TN values were significantly lower than those of the randomly generated assemblages (TN = 4.10, SES = -2.95, $p = 0.001$, Fig. 3c), probably due to an increase in species richness over time.

Similarly, the observed TT value along the entire period (2002–2015) did not significantly differ from the randomly generated assemblages (TT = 0.37, SES = -1.56, $p > 0.05$, Fig. 4a). However, in the comparisons 2002–2003 and 2003–2015, the observed TT values were significantly higher than those of the randomly generated assemblages (TT = 0.65, SES = 3.57, $p = 0.001$, Fig. 4b; TT = 0.60, SES = 2.58, $p = 0.007$, Fig. 4c, respectively).

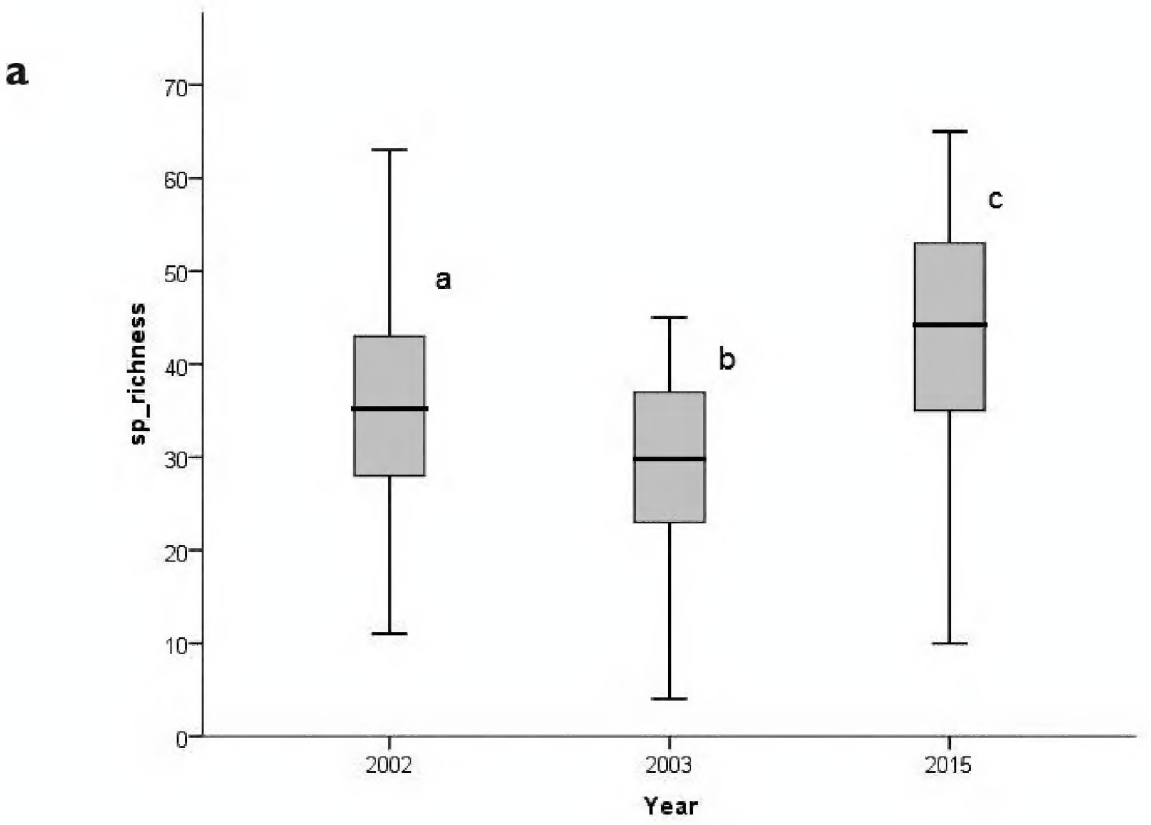

Figure 2. Mean values and variability of species richness per plot **a** for all the ecosystems, and **b** according to the two main ecosystems, i.e. grasslands (blue colour) and forests (green colour). Different letters indicate significant differences in mean values across time following post-hoc test results.

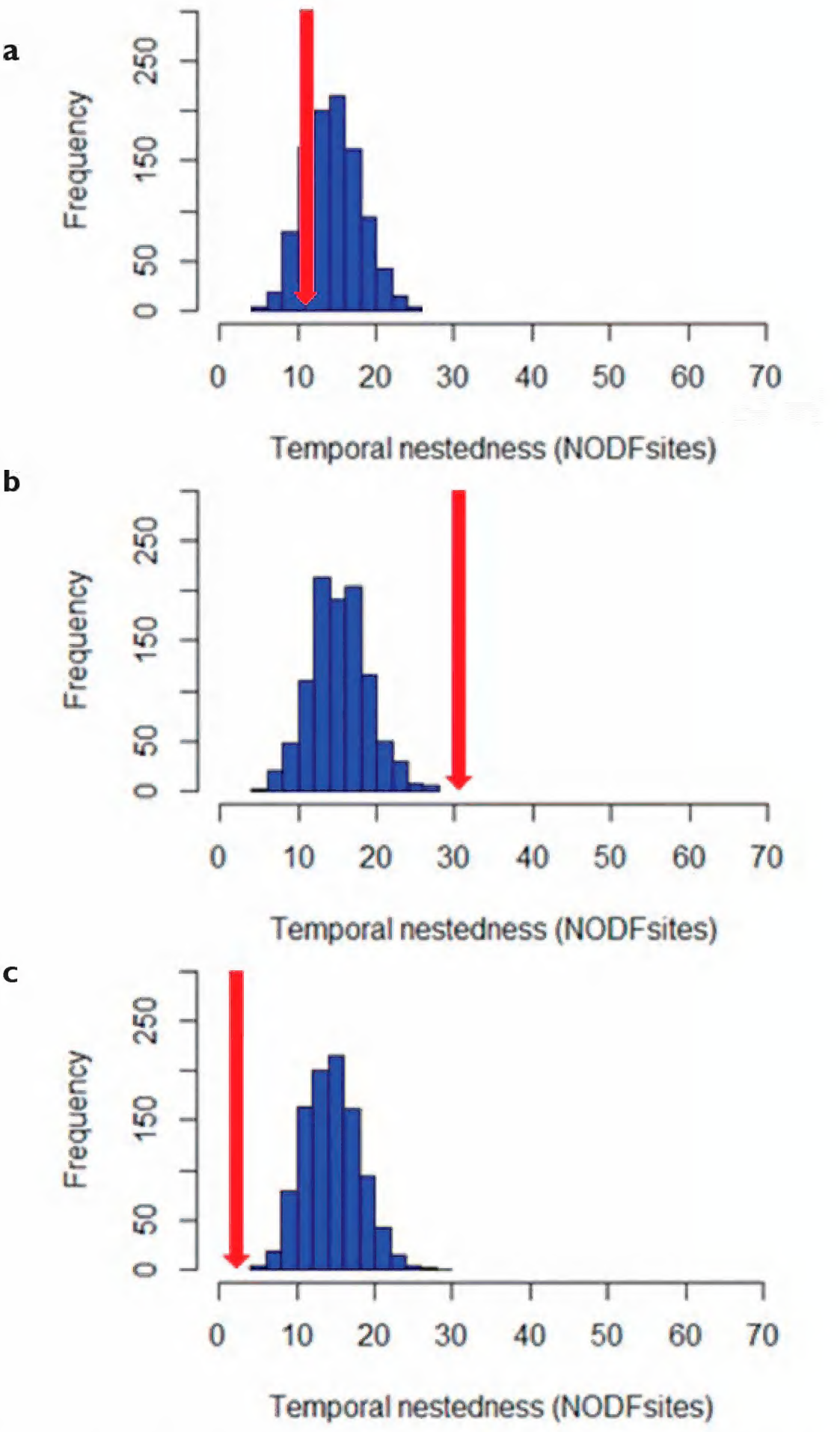

Figure 3. Temporal nestedness observed for **a** 2002 vs 2015 surveys **b** 2002 vs 2003 surveys and **c** 2003 vs 2015 surveys. The arrow indicates the position of the observed temporal nestedness value (NODFsites) with respect to the scores of the random loop (999 random nestedness values).

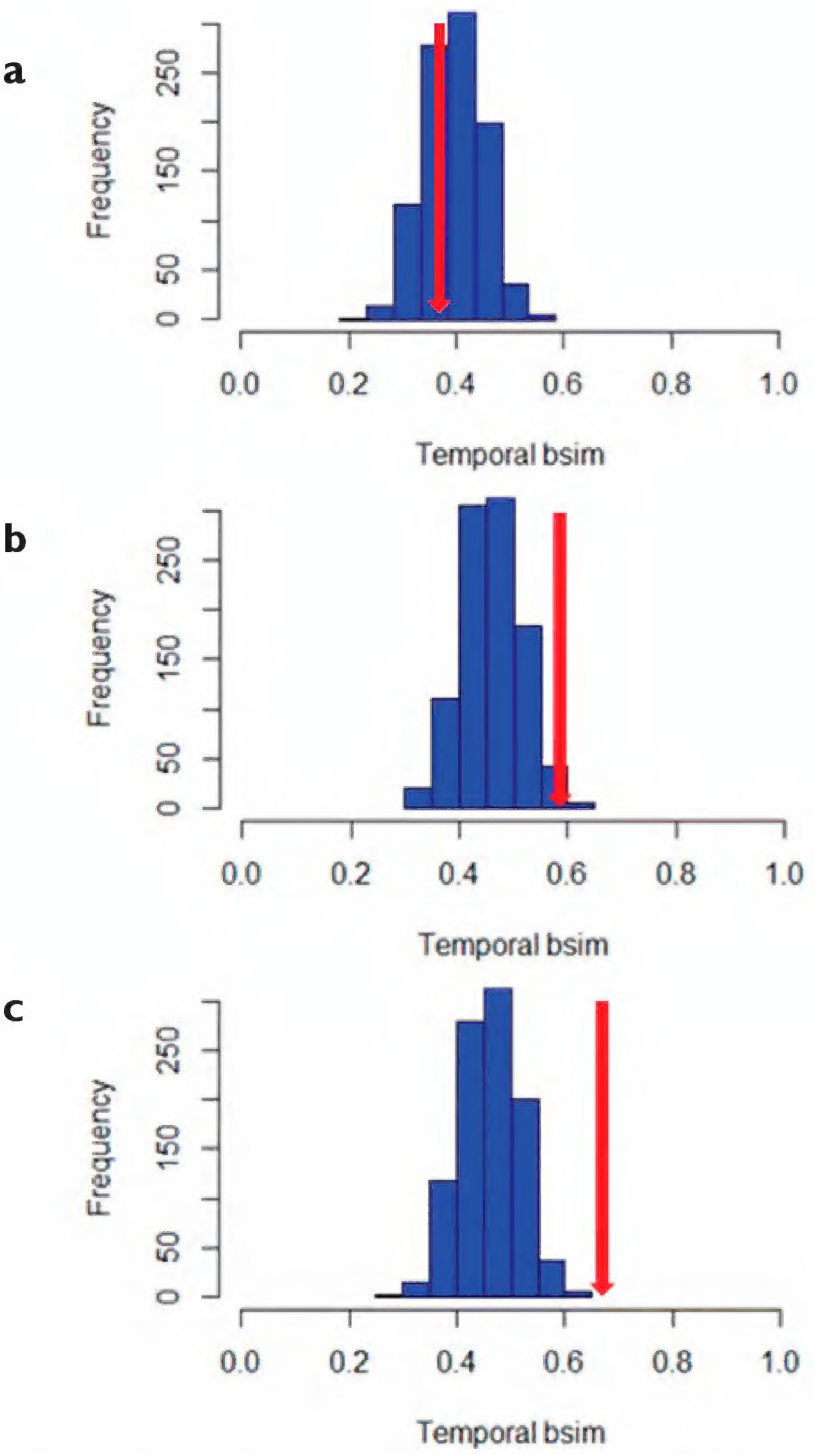

Figure 4. Temporal turnover observed for **a** 2002 vs 2015 surveys **b** 2002 vs 2003 surveys and **c** 2003 vs 2015 surveys. The arrow indicates the position of the observed temporal turnover value (bsim) with respect to the scores of the random loop (999 random turnover values).

Discussion

Our results showed significantly different values of species richness at the plot level, confirming our prediction, with the year 2003 having the lowest species richness value. The three study years showed different climatic conditions: the vegetative season of 2003 had 47% less precipitation than that of 2002, and 57% less than that of 2015. Focusing on spring, the year 2003 was particularly dry, registering 42% and 73% less precipitation than that measured for the spring seasons of 2002 and 2015, respectively (Source: ASSAM, Marche Region). Seasonal distribution of precipitation is known to influence processes triggering plant survival and growth, such as tiller production,

root-shoot biomass, root depth, canopy leaf area, stomatal conductance and photosynthesis (Zeppel et al. 2014). In particular, studies demonstrated the effect of spring climatic conditions on species growth rate (Battipaglia et al. 2009), plant functional traits (Wellstein et al. 2017), ecosystem productivity (Chelli et al. 2016), species cover and richness (Chiarucci et al. 2003) in sub-Mediterranean systems where water availability represents a strong limiting factor (Chelli et al. 2017). Our results, using a probabilistic sampling design and a diachronic dataset, confirm a significant reduction of species richness at plot level as a quick response to drought. Unfortunately, we did not repeat the sampling in the years immediately after the dry spell of 2003, thus we cannot infer information about species richness related to the timing of recovery.

Exploring in detail the pattern of species richness variation across the two main systems of the Nature Reserve (grasslands and forests), our results showed that forest ecosystems maintain a certain stability in terms of species richness at plot level over time, according to our predictions. The structure of ecosystems is known to influence local microclimate which in turn can buffer macroclimate variability. This is the case with forest ecosystems, where the canopy cover regulates temperature, light availability, wind speed and soil moisture, affecting ecological processes of the understory layer (Cervellini et al. 2017), including plant growth and survival, soil respiration and nutrient cycling (Chen et al. 1999). In addition, forest understories are mainly composed of perennial species, which are less sensitive to interannual climate variability. On the contrary, in line with our results, several studies reported yearly fluctuation of species richness in parallel with seasonal climatic conditions in Mediterranean and sub-Mediterranean grasslands (Chiarucci et al. 2003; Elmendorf and Harrison 2009). However, the relationship between these patterns and weather conditions is still not well understood. These systems are characterised by the occurrence of several annual species that can complete their life cycle rapidly during periods when conditions are favourable and survive unfavourable conditions (including drought) as seeds. In the studied grasslands we recorded during the dry 2003 the absence of many annual species, for example *Poa annua*, *Linum catharticum*, *Bromus hordeaceus*, *Alyssum minus*, *Myosotis* sp., *Bupleurum baldense*, and *Polygonum arenastrum*, all of which were present in the 2002 and 2015 relevés. On the contrary, perennial species occurring in grassland systems of the study area were more stable over time. They are mainly clonal and equipped with different types of belowground storage organs (e.g. rhizomes and tap roots, Halassy et al. 2005), and thus can deal with climate variability by using their stored energy and adjusting their growth rate (Wellstein et al. 2017). Instead, many perennial non clonal forbs and shrubs have deep rooting systems (e.g. *Astragalus sempervirens*, Wellstein et al. 2013), which are widely thought to buffer the plants from drought because they allow access to deeper water (West et al. 2012).

Along the entire monitoring period (2002–2015), the species' compositional changes were random, i.e. not related to significant patterns of species impoverishment or species turnover, indicating a certain stability in terms of species composition without tendencies due to the legacy of the previous land use. This result highlights that in the study area, the time interval of 13 years was not wide enough to detect successional effects in terms of compositional changes. However, comparisons including the dry year

2003 showed significant patterns of both species' impoverishment and turnover, with important implications in terms of community assembly. Our data probably illustrates how interannual environmental variation can generate communities that are nested across time: common species are always present, while rare species disappear in bad years such as the dry 2003, largely as a function of their relative abundances (Elmendorf and Harrison 2009). In parallel, we also observed a significant species turnover with respect to 2003, in line with Ulrich et al. (2017) who found that species turnover was expected to be important only in the case of high environmental heterogeneity (both spatially and temporally). This result probably depends on two mechanisms. First, the occurrence of species that can benefit from dry conditions (bad-year specialists, Elmendorf and Harrison 2009). This is in line with the findings of Wellstein et al. (2017) who showed how some grass species in sub-Mediterranean ecosystems (e.g. *Bromus erectus* and the endemic *Sesleria nitida*) surprisingly benefited from extreme drought. Second, the reduced competitive strength of dominant species due to drought (e.g. clonal grasses forming high-density tussocks or dense carpets, Wellstein et al. 2014), thus enabling more subordinate species to coexist. However, the contemporary occurrence of both species replacement and species loss processes resulted from this research in the dry year calls for more detailed diachronic studies. Here, experimental approaches manipulating water availability (see Jentsch et al. 2007; Jentsch and Beierkuhnlein 2008; Chelli et al. 2017) could be key to assessing fine-scale mechanisms of community assembly.

Conclusion

Thanks to the long-term approaches, LTER sites can provide unique insights potentially useful for managers of protected areas where there is the urgent need of monitoring species diversity over time. Our approach demonstrated the usefulness of long-term plant diversity monitoring programmes based on probabilistic sampling designs for the study of species richness and patterns of species assemblages over time in a protected area. Although we did not study environmental drivers directly, we showed how interannual climate variability could play a key role in shaping plant species richness and assemblages over time. Forest systems seem to buffer the impact of short-term variations, at least in terms of species richness. Nevertheless, the effects of interannual climate variability on species interactions and community structure are only beginning to be evaluated empirically, and more studies are needed. Accordingly, we suggest several considerations for future studies. First, spatial scale should be taken into account, since changes in species richness across vegetation types varies with scale of observation (Peet et al. 2014); moreover, a characteristic scale of spatial assemblages can emerge along the dynamics (e.g., Bartha et al. 2004). Second, plant cover values should be used as an indicator of species abundance, together with information on species presence/absence, to better assess community changes at multiple scales (Chiarucci et al. 2003). Third, approaches based on plant traits or functional groups can likely provide novel insights about the drivers of species diversity changes and ecosystem functioning (Suding et al. 2008).

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